PATENT
Attorney Docket No.: 408445

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ethan R. Signer et al.	Confirmation No.: 4053
Application No.: 09/879,329	Group No.: 1633
Filed: June 12, 2001	Examiner: Woitach, Joseph T.
For: REMOVAL OF SELECTABLE MARKERS FROM TRANSFORMED CELLS	

December 11, 2008

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APPEAL BRIEF

Dear Sir:

In accordance with 37 C.F.R. §41.37, and fully responsive to the Final Office Action dated February 12, 2008, Appellant hereby files the Appeal Brief in support of the Appeal in the above-identified matter U.S. Patent Application Serial Number 09/879,329 (hereinafter the '329 Application). A Notice of Appeal, with the appropriate small entity fee of $255 as required by 37 C.F.R. §§41.31, 41.20(b)(1), was filed on July 11, 2008. This appeal brief is also submitted along with authorization to charge the required fees for appeal brief and three month extension of time to Deposit Account No. 12-0600.

(1) Real party in interest

The real parties in interest for this appeal are Missouri Soybean Merchandising Council and The Curators of the University of Missouri.

(2) Related appeals and interferences

No other pending appeals or interferences are currently known to Appellant that will directly affect, be directly affected by, or have a bearing on the decision to be rendered by the Board of Patent Appeals and Interferences in the instant appeal.

(3) Status of claims

Claims 1-7, 10-16, 18 and 21 are at issue in this appeal. Claims 8-9, 19-20 are allowed. Claims 1-7, 10-16, 18 and 21 were rejected in the last Office Action dated February 12, 2008. Claims 1-20 were in the original application filed on June 12, 2001. Claim 21 was added in the response to Office Action submitted on December 23, 2005. Claims 1-7, 10-16, 18 and 21 stand rejected as follows:

Claims 1-7, 10-16, 18 and 21 stand rejected under 35 U.S.C. 103(a) as being obvious over U.S. Patent No. 6,984,774 issued to Peterson (hereinafter "Peterson"), in view of U.S. Patent No. 6,534,315 issued to Bauer (hereinafter "Bauer") (Examiner referred to Bauer as U.S. Patent No. 6,984,774 in the last Office Action, Appellant assumes that Examiner meant to say U.S. Patent No. 6,534,315. Confirmation from the Examiner is respectfully requested), and further in view of U.S. Patent Publication No. 2002/0035739 by Lassner (hereinafter "Lassner"). Appellant respectfully traverses this rejection and requests withdrawal of same.

(4) Status of amendments

The '329 Application was originally filed on June 12, 2001. On February 12, 2008, a final office action was mailed, prompting this appeal. A Notice of Appeal was filed on July 11, 2008. No amendments have been submitted or entered after the final Office Action of February 12, 2008.

(5) Summary of claimed subject matter

Claims 1-21 are directed to materials and methods for transforming plants and for removing marker gene(s) from the transformed plants. Claims 1, 4, and 21 are the only independent claims on appeal.

Claim 1 recites a genetic construct for use in transforming plant cells. The construct comprises

(a) a positive selectable marker gene that when transformed into the host plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

(b) a negative selectable marker gene that when rendered operable in the host plant cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

(c) two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with the direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b).

Referring to the Specification as originally filed, Paragraphs 6-7 on pages 2-3 describe the construct with a positive marker and a negative marker, which are flanked by two direct repeats. Figures 1A and 1B show that the direct repeats immediately flank the two marker genes and that each direct repeat comprises a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells. Paragraph 32 on page 9 of the original specification teaches that a gene of interest may include nucleic acids encoding viral, parasitic, tumor, bacterial, or other known immunogens which may be expressed in plants. Paragraph 35 on page 10 describes the presence of two copies of the gene of interest flanking the marker genes.

Claim 4 recites a method for removing selectable marker genes from transformed plant cells. The method of Claim 4 recites the steps of:

(a) transforming plant cells with a genetic construct that includes

a positive selectable marker gene that when transformed into the plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

a negative selectable marker gene that when rendered operable in the plant cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with one of said direct repeats immediately flanking the positive selectable marker gene and the other of said direct repeats immediately flanking said negative selectable marker gene,

to provide transformed plant cells;

(b) culturing the transformed plant cells of (a) on a positive selective medium,

(c) transferring the transformed plant cells in (b) onto a negative selective medium, and

(d) selecting the transformed plant cells that grow on the negative selective medium wherein the selected transformed plant cells that grow on the negative selective medium contain the gene sequence of interest but neither the positive selectable marker sequence nor the negative selectable marker sequence.

Referring to the Specification as originally filed, Paragraphs 5-7 on pages 2-3 describe a construct with a positive marker and a negative marker, which are flanked by two direct repeats. Paragraphs 5-7 on pages 2-3 also teaches introduction of this construct into a plant cell, and selecting for plant transformant using the positive selectable marker. Paragraph 8 on page 3 teaches the recombination events that remove the marker genes and the selection of the plant transformants using negative selectable medium. Figures 1A and 1B show that the direct repeats immediately flank the two marker genes and that each direct repeat comprises a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells. Paragraph 32 on page 9 of the original specification teaches that a gene of interest may include nucleic acids encoding viral, parasitic, tumor, bacterial, or other known immunogens which may be expressed in plants. Paragraph 35 on page 10 describes the presence of two copies of the gene of interest flanking the marker genes.

Claim 21 recites a genetic construct for use in transforming cells, wherein the construct comprises

(a) a positive selectable marker gene that when transformed into the cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

(b) a negative selectable marker gene that when rendered operable in the cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

(c) two direct repeats of a gene of interest in a host cell, the direct repeats being effective for use in recombination with the genome of the host cells, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, said direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b),

wherein the negative selectable marker gene is CodA.

Referring to the Specification as originally filed, Paragraphs 6-7 on pages 2-3 describe the construct with a positive marker and a negative marker, which are flanked by two direct repeats. Figures 1A and 1B show that the direct repeats immediately flank the two marker genes and that each direct repeat comprises a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells. Paragraph 32 on page 9 of the original specification teaches that a gene of interest may include nucleic acids encoding viral, parasitic, tumor, bacterial, or other known immunogens which may be expressed in plants. Paragraph 35 on page 10 describes the presence of two copies of the gene of interest flanking the marker genes. Figure 1B also shows that the negative selectable marker gene may be CodA.

(6) Grounds for rejections to be reviewed on appeal

Whether Claims 1-7, 10-16, 18 and 21 are unpatentable under 35 U.S.C. 103(a) as being obvious over Peterson, in view of Bauer, and further in view of Lassner.

(7) Arguments

Rejections of Claims 1-7, 10-16, 18 and 21.

Claims 1-7, 10-16, 18 and 21 stand rejected under 35 U.S.C. 103(a) as being obvious over U.S. Patent No. 6,984,774 issued to Peterson (hereinafter "Peterson"), in view of U.S. Patent No. 6,534,315 issued to Bauer (hereinafter "Bauer") (Examiner referred to Bauer as U.S. Patent No. 6,984,774 in the last Office Action, Appellant assumes that Examiner meant to say U.S. Patent No. 6,534,315. Confirmation from the Examiner is respectfully requested), and further in view of U.S. Patent Publication No. 2002/0035739 by Lassner (hereinafter "Lassner"). Appellant respectfully traverses the rejection because the cited references taken together do not teach or suggest Appellant's invention as claimed and it would not have been be obvious for one of ordinary skill in the art to modify the teachings of the cited references in order to arrive at Appellant's invention.

Obviousness is a question of law based on underlying factual inquiries. The factual inquiries enunciated by the U.S. Supreme Court in *KSR Int'l C. v. Teleflex, Inc.*, 127 S. Ct. 1727, 82 USPQ2d 1385 (2007) include the *Graham* factors of determining the scope and content of the prior art, ascertaining the differences between the claimed invention and the prior art, and resolving the level of ordinary skill in the pertinent art.

Once the *Graham* factual inquiries are resolved, the Examiner must explain why the difference(s) between the cited references and the claimed invention would have been obvious to one of ordinary skill in the art. The rationale used must be a permissible rationale. The USPTO has promulgated examination guidelines for determining obviousness in view of *KSR* in M.P.E.P. §2143(A)-(G). These *KSR* Guidelines enumerate permissible rationales and the findings of fact that must be made under the particular rationale.

(i) The teaching of the cited references

Peterson relates to "methods and materials to induce homologous recombination in a plant, comprising introducing a recombination construct to a plant and making available to the plant a transposase, so as to induce recombination." *See* abstract of Peterson. Peterson discloses a construct containing a Ds element flanked by direct

repeats or overlapping sequences. Col. 3, lines 25-42 of Peterson. Peterson further teaches that the construct may optionally contain a selectable marker. Col. 3, lines 43-44 of Peterson. The only example provided by Peterson is a construct with a Ds element located between two partially overlapping non-functional segments of the GUS gene. Col. 9, lines 35-39; *see also* Fig. 1 of Peterson.

Bauer does not teach direct repeats that can lead to expression of the peptide encoded by the direct repeat. On the contrary, Bauer teaches how the direct repeats should be rendered noncoding. *See e.g.*, lines 45-48, Col. 4 and lines 22-28, Col. 7 of Bauer, stating "... made noncoding by any appropriate means such as changing the reading frame or the introduction of stop codons."

Lassner relates to methods for identifying plant disease resistant genes and is only relied upon by the Examiner to show that a negative selectable marker can be used in plant engineering.

(ii) Substantial differences exist between the cited references and Appellant's invention.

Applicant respectfully submits that substantial differences exist between the three cited references and the present invention for the following reasons:

First, although Peterson teaches that the construct may contain two direct repeats, Peterson never specifically teaches or suggests that each of the two repeats comprises a nucleic acid sequence encoding a peptide that is capable of being expressed in the plant. Indeed, Peterson states that the two direct repeats can encode a gene product when recombined, but never mentions that each direct repeat alone comprises a nucleic acid sequence encoding a peptide that is capable of being expressed in the plant, as is taught and claimed by Appellant.

This difference between the present invention and Peterson likely stems from the different design of the constructs. The present application discloses a construct with two copies of the same gene, one in each direct repeat. By contrast, Peterson's construct uses two "non-functional," or truncated fragments of a gene as the direct repeats. *See* Col. 9, lines 35-39. More specifically, one direct repeat taught by Peterson is an N-terminal fragment, while the other direct repeat is a C-terminal fragment of the same gene. *Id.*; *see also* Fig. 1 of Peterson. These two fragments shares a "partially overlapping"

sequences. *Id.* While Appellant's claims require that each of the two direct repeats can independently encode a peptide that is capable of being expressed in the plant cell, Peterson never requires that both direct repeats encode a peptide.

Second, even if we assume that the direct repeats of Peterson were the same as those of the instant invention, the direct repeats of Peterson do not immediately flank the positive and negative markers, which is required by Appellant's claims 1, 4 and 21. According to the specification and Fig. 1 of Peterson, the Ds element is located between the positive marker gene and the direct repeat. Therefore, the marker gene of Peterson is not immediately flanked by the direct repeats on both sides, as is required by Appellant's claims. The Ds element is required for operation of the invention disclosed in Peterson and cant not be readily removed without rendering the Peterson invention inoperable. *See e.g.*, Col. 2, lines 46-49 of Peterson, stating that the "invention is directed to the unexpected finding that overlapping foreign gene sequences containing a maize Ds element can be induced to undergo homologous recombination upon introduction of the maize Ac transposase."

(iii) It would not have been obvious to modify the teaching of the cited references to arrive at Appellant's invention.

Not only does the teaching of the cited references substantially different from Appellant's invention, but the differences are not such that it would have been obvious for one of ordinary skill in the art to modify the teaching of the cited references to arrive at Appellant's invention.

First, Bauer is not to be combined with Peterson because doing so would render the prior art unsatisfactory for its intended purpose or change the principle of operation of at least one of the references. In discussing obviousness rejection by combining multiple references, the MPEP states that a "proposed modification cannot render the prior art unsatisfactory for its intended purpose or change the principle of operation of a reference." MPEP 2145. The Examiner relies on Bauer to teach a construct with a positive and negative selectable markers flanked by two direct repeats, one on each side. The Examiner further relies on Peterson to disclose two direct repeats that encode a peptide capable of being expressed in the plant. In doing so, the Examiner ignores the fact that Bauer can not be combined with Peterson because Bauer specifically teaches

that the direct repeat sequence is noncoding, i.e., not translated into the form of a peptide. To combine Bauer with Peterson would render Bauer unsatisfactory for its intended purpose because one of the objectives of Bauer is to leave behind no direct repeat sequence that encodes a peptide after excision. *See e.g.*, Col. 4, lines 35-48 of Bauer.

Secondly, even if the teachings of Peterson, Bauer and Lassner are combined, Appellant's claimed invention is not obvious because the Examiner has not provided any rationale as to why one of ordinary skill would be motivated to modify the direct repeats of Peterson to obtain Appellant's claimed construct. As explained above, Peterson never mentions that each direct repeat encodes a peptide. Rather, Peterson teaches that the direct repeats encode a peptide after recombination. This is in contrast to Appellant's invention where each direct repeat comprises a nucleic acid sequence that as so that the modified repeats would each encode a peptide without requiring recombination. The Examiner has not established why one of skill in the art would have found it obvious to modify the direct repeat of Peterson in order to arrive at Appellant's construct.

Last but not the least, the Examiner has not provided any motivation either in the references or in the common knowledge of one of ordinary skill in the art to remove the Ds element in Peterson's construct. As explained above, the Ds element is sitting between the selectable marker and one of the direct repeats. In order to arrive at Appellant's claimed invention, the Ds element need to be removed so that the selectable markers are immediately flanked by two direct repeats with each direct repeat encoding a peptide capable of being expressed in the plant cells. However, removing the Ds element would have rendered the Peterson invention inoperable.

Appellant recognizes that the references need to be considered as a whole, however, it is the conflicting teachings of the references that would discourage one of ordinary skill in the art to combine their teachings. Even if one is to combine these references, one would not find it obvious to reconcile these conflicting teachings in order to arrive at Appellant's claimed invention. As the Supreme Court warned against in Graham and reiterated in KSR, a fact finder must resist the temptation to read into the prior art the teachings of the invention at issue. Appellant respectfully submits that the Examiner has not established that one of ordinary skill in the art would be motivated to

modify the conflicting teachings of the three cited references in a manner claimed by Appellant without slipping into the use of hindsight.

Thus, because substantial differences exist between the cited references and Appellant's claimed invention, and because such differences would not have been obvious to one of skill in the art at the time of Appellant's invention, Appellant's invention is not rendered obvious by the cited references.

The following commentary is provided with respect to the individual claims:

Claim 1

Claim 1 recites a genetic construct for use in transforming host plant cells:

1. A genetic construct for use in transforming host plant cells, comprising:
a. a positive selectable marker gene that when transformed into the host plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,
b. a negative selectable marker gene that when rendered operable in the host plant cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and
c. two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with the direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b).

In regard to Claim 1, the cited references do not teach or suggest two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with the direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b).

Claim 2

Claim 2 recites the construct of Claim 1 wherein negative selectable marker gene is CodA. Claim 2 depends from Claim 1 and necessarily incorporate all limitations of Claim 1.

Claim 3

Claim 3 recites the genetic construct of claim 2 wherein the positive selectable marker gene is NPTII, BAR, PAT or EPSP synthase.

Claim 6

Claim 6 depends from Claim 1 and necessarily incorporate all limitations of Claim 1. Claim 6 also adds additional limitations to Claim 1:

6. (Previously Presented) The genetic construct of claim 1, wherein said construct comprises a polynucleotide sequence in the 5' to 3' (right to left) direction:
a. a gene sequence of interest,
b. a positive selectable marker sequence,
c. a negative selectable marker sequence and
d. a repeat of the gene sequence of interest in (a) above.

Claim 6 calls for a gene sequence of interest and a repeat of the same gene sequence of interest. Peterson's direct repeats are both truncated fragment of a gene sequence but not a gene sequence of interest. Moreover, Peterson's two repeats only have partial overlap and therefore do not contain a repeat of the same gene sequence of interest. Neither Bauer nor Lassner discloses a gene sequence of interest and a repeat of the same gene sequence of interest.

Claim 7

Claim 7 recites the genetic construct of claim 6 wherein the negative selectable marker sequence is CodA. Claim 7 depends from Claim 6 and necessarily incorporate all limitations of Claim 6.

Claim 10

Claim 10 recites the genetic construct of claim 1 wherein the construct comprises, in the 5' to 3' direction (left to right), the formula GI-PS-NS-GI, wherein GI represents a gene of interest, PS represents a positive selectable marker gene and NS represents a negative selectable marker gene. Claim 10 requires that two identical copies of GI immediately flank the positive and negative selectable markers. This limitation is neither taught nor suggested by the cited references.

Claim 11

Claim 11 recites the genetic construct of claim 10 wherein the negative selectable marker sequence is CodA. Claim 11 depends from Claim 10 and necessarily incorporate all limitations of Claim 10.

Claim 12

Claim 12 recites the genetic construct of claim 1 wherein the construct comprises, in the 5' to 3' direction (left to right), the formula GI-NS-PS-GI, wherein GI represents a gene of interest, PS represents a positive selectable marker gene and NS represents a negative selectable marker gene. Claim 12 requires that two identical copies of GI immediately flank the positive and negative selectable markers. This limitation is neither taught nor suggested by the cited references.

Claim 13

Claim 13 recites the genetic construct of claim 12 wherein the negative selectable marker sequence is CodA. Claim 13 depends from Claim 12 and necessarily incorporate all limitations of Claim 12.

Claim 14

Claim 14 recites the genetic construct of claim 1 wherein the construct comprises, in the 5' to 3' direction (left to right), the formula AGx-GI-PS-NS-GI-AG'y, wherein AG and AG' represent additional genes of interest, x represents an integer of 1 or larger, y represents an integer of 0 or larger, GI represents a gene of interest, NS represents a negative selectable marker gene, and PS represents a positive selectable marker gene. Claim 14 requires that two identical copies of GI immediately flank the positive and negative selectable markers. This limitation is neither taught nor suggested by the cited references.

Claim 15

Claim 15 recites the genetic construct of claim 14 wherein the genes represented by AG and AG' are never the same. Claim 15 depends from Claim 14 and necessarily incorporate all limitations of Claim 14.

Claim 16

Claim 16 recites the genetic construct of claim 14 wherein the negative selectable marker sequence is CodA. Claim 16 depends from Claim 14 and necessarily incorporate all limitations of Claim 14.

Claim 21

Claim 21 recites a genetic construct for use in transforming cells:

21. (Previously presented) A genetic construct for use in transforming cells, comprising:

a. a positive selectable marker gene that when transformed into the cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

b. a negative selectable marker gene that when rendered operable in the cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

c. two direct repeats of a gene of interest in a host cell, the direct repeats being effective for use in recombination with the genome of the host cells, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, said direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b),

wherein the negative selectable marker gene is CodA.

In regard to Claim 21, the cited references do not teach or suggest two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with the direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b).

Claim 4

Claim 4 recites a method for removing selectable marker genes from transformed plant cells:

4. A method of removing selectable marker genes from transformed plant cells which comprises:

a. transforming plant cells with a genetic construct that includes

a positive selectable marker gene that when transformed into the plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

a negative selectable marker gene that when rendered operable in the plant cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with one of said direct repeats immediately flanking the positive selectable marker gene and the other of said direct repeats immediately flanking said negative selectable marker gene,

to provide transformed plant cells;

b. culturing the transformed plant cells of (a) on a positive selective medium,

c. transferring the transformed plant cells in (b) onto a negative selective medium, and

d. selecting the transformed plant cells that grow on the negative selective medium wherein the selected transformed plant cells that grow on the negative selective medium contain the gene sequence of interest but neither the positive selectable marker sequence nor the negative selectable marker sequence.

In regard to Claim 4, the cited references do not teach or suggest the use of two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with the direct repeats immediately flanking the positive and negative selectable marker genes, as recited in Step (a).

Claim 5

Claim 5 recites the method of claim 4 wherein the negative selectable marker gene is CodA. Claim 5 depends from Claim 4 and necessarily incorporate all limitations of Claim 4.

Claim 18

Claim 18 recites the method of claim 4 wherein the plant cell is a corn, soybean, cotton, wheat, canola, tobacco, Arabidopsis, rice, safflower or sunflower cell. Claim 18 depends from Claim 4 and necessarily incorporate all limitations of Claim 4.

The method of claim 4 wherein the plant cell is a corn, soybean, cotton, wheat, canola, tobacco, Arabidopsis, rice, safflower or sunflower cell.

(8) Claims appendix.

Appellant has enclosed a copy of Claims 1-21 including those claims that have been cancelled or withdrawn as an appendix hereto.

(9) Evidence appendix.

Not applicable.

(10) Related proceedings appendix.

Not applicable.

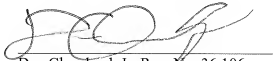
CONCLUSION

Appellant respectfully requests the Honorable Board of Appeals reverse the Examiner in the rejections of Claims 1-7, 10-16, 18 and 21 under 35 U.S.C. § 103(a). Appellant respectfully solicits allowance of Claims 1-7, 10-16, 18 and 21.

Other than the costs for this appeal brief and the cost for extension of time, no further fees are deemed due in connection with this matter. However, the Commissioner is hereby authorized to charge any fees which may be due in this matter from Deposit Account Number 12-0600.

Respectfully submitted,

LATHROP & GAGE L.C.



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Claims Appendix

1. (Previously presented) A genetic construct for use in transforming host plant cells, comprising:

a. a positive selectable marker gene that when transformed into the host plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

b. a negative selectable marker gene that when rendered operable in the host plant cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

c. two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with the direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b).

2. (Previously Presented) The genetic construct of claim 1 wherein the negative selectable marker gene is CodA.

3. (Previously Presented) The genetic construct of claim 2 wherein the positive selectable marker gene is NPTII, BAR, PAT or EPSP synthase.

4. (Previously presented) A method of removing selectable marker genes from transformed plant cells which comprises:

a. transforming plant cells with a genetic construct that includes

a positive selectable marker gene that when transformed into the plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

a negative selectable marker gene that when rendered operable in the plant cells hinders growth on a negative selective medium that is complementary to the

negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

two direct repeats of a gene of interest, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, with one of said direct repeats immediately flanking the positive selectable marker gene and the other of said direct repeats immediately flanking said negative selectable marker gene,

to provide transformed plant cells;

b. culturing the transformed plant cells of (a) on a positive selective medium,

c. transferring the transformed plant cells in (b) onto a negative selective medium, and

d. selecting the transformed plant cells that grow on the negative selective medium wherein the selected transformed plant cells that grow on the negative selective medium contain the gene sequence of interest but neither the positive selectable marker sequence nor the negative selectable marker sequence.

5. (Original) The method of claim 4 wherein the negative selectable marker gene is CodA.

6. (Previously Presented) The genetic construct of claim 1, wherein said construct comprises a polynucleotide sequence in the 5' to 3' (right to left) direction:

a. a gene sequence of interest,

b. a positive selectable marker sequence,

c. a negative selectable marker sequence and

d. a repeat of the gene sequence of interest in (a) above.

7. (Previously Presented) The genetic construct of claim 6 wherein the negative selectable marker sequence is CodA.

8. (Previously presented) A method of removing selectable marker genes from transformed plant cells which comprises:

a. transforming plant cells with a genetic construct to form T0 transformants, wherein the genetic construct includes

a positive selectable marker gene that when transformed into the plant cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

a negative selectable marker gene that when rendered operable in the plant cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

two direct repeats of a gene of interest, the direct repeats being effective for use in recombination with the genome of the host plant cells, one of said direct repeats immediately flanking the positive selectable marker gene and the other of said direct repeats immediately flanking said negative selectable marker gene,

b. culturing the plant cells of (a) on a positive selective medium,

c. selecting T0 transformant cells that grow on the positive selective medium,

d. regenerating a fertile T0 plant from the T0 transformant cells whereby T1 seed is formed,

e. collecting the T1 seed from the T0 plant or the seed from a subsequent Tn generation plant wherein n is a whole number greater than one,

f. germinating the T1 seeds or Tn seeds on a negative selective medium to form seedlings, and

g. selecting the seedlings that grow on the negative selective medium wherein the selected seedlings contain the gene sequence of interest but neither the positive selectable marker sequence nor the negative selectable marker sequence.

9. (Original) The method of claim 8 wherein the negative selectable marker gene is CodA and the negative selective medium comprises 5-fluorocytosine.

10. (Previously Presented) The genetic construct of claim 1, wherein said construct comprises, in the 5' to 3' direction (left to right), the formula:

GI-PS-NS-GI

wherein GI represents a gene of interest, PS represents a positive selectable marker gene and NS represents a negative selectable marker gene.

11. (Previously Presented) The genetic construct of claim 10 wherein NS is CodA.

12. (Previously Presented) The genetic construct of claim 1, wherein said construct comprises, in the 5' to 3' direction (left to right), the formula:

GI-NS-PS-GI

wherein GI represents a gene of interest, NS represents a negative selectable marker gene, and PS represents a positive selectable marker gene.

13. (Previously Presented) The genetic construct of claim 12 wherein NS is CodA.

14. (Previously Presented) The genetic construct of claim 1, wherein said construct comprises, in the 5' to 3' direction (left to right), the formula:

AGx-GI-PS-NS-GI-AG'y

wherein AG and AG' represent additional genes of interest, x represents an integer of 1 or larger, y represents an integer of 0 or larger, GI represents a gene of interest, NS represents a negative selectable marker gene, and PS represents a positive selectable marker gene.

15. (Previously Presented) The genetic construct of claim 14 wherein the genes represented by AG and AG' are never the same.

16. (Previously Presented) The genetic construct of claim 14 wherein the NS is CodA.

17. (Canceled).

18. (Previously presented) The method of claim 4 wherein the plant cell is a corn, soybean, cotton, wheat, canola, tobacco, Arabidopsis, rice, safflower or sunflower cell.

19. (Original) The method of claim 8 wherein the plant cell is a monocot or dicot cell.

20. (Previously Presented) The method of claim 19 wherein the plant cell is a corn, soybean, cotton, wheat, canola, tobacco, Arabidopsis, rice, safflower or sunflower cell.

21. (Previously presented) A genetic construct for use in transforming cells, comprising:

a. a positive selectable marker gene that when transformed into the cells facilitates growth on a positive selective medium that is complementary to the positive selective marker gene,

b. a negative selectable marker gene that when rendered operable in the cells hinders growth on a negative selective medium that is complementary to the negative selectable marker, the negative selectable marker being different in kind from the positive selectable marker, and

c. two direct repeats of a gene of interest in a host cell, the direct repeats being effective for use in recombination with the genome of the host cells, each direct repeat comprising a nucleic acid sequence encoding a peptide, wherein the peptide is capable of being expressed in said plant cells, said direct repeats immediately flanking the positive and negative selectable marker genes of (a) and (b),

wherein the negative selectable marker gene is CodA.

Evidence Appendix

Not Applicable.

Related Proceedings Appendix

Not applicable.